Cisplatin

Molecular formula: $Cl_2H_6N_2Pt$
Molecular weight: 300.05
CAS Registry No.: 15663-27-1
Merck Index: 2378
Lednicer No.: 4 15-17

SAMPLE

Matrix: blood
Sample preparation: Filter (Amicon Centriflo CF 50A) while centrifuging at 2500 rpm, inject a 25 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: Guard-Pak C18 (Waters)
Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: 10 mM pH 4.6 Sodium acetate buffer containing 5 mM heptanesulfonic acid
Flow rate: 1 (A), 0.5 (B, C)
Injection volume: 25
Detector: E, HMDE 174 A/310 (EG & G, Princeton Applied Research), hanging mercury drop electrode, polarographic cell, operated at 100 mV vs Ag/AgCl in HMDE mode with small drop size (A); E, LC4B (Bioanalytical Systems, West Lafayette IN) with a Model TL-9A thin-layer transducer cell, Au/Hg amalgam working electrode at -100 mV, reference electrode Ag/AgCl (B); atomic absorption of fractions, IL 951-555 systems (Allied Analytical Systems, Lexington MA), 265.95 nm, band-pass 0.5 nm, lamp current 10 mA (C)

CHROMATOGRAM

Retention time: 1.6
Limit of detection: 62 ng/mL

KEY WORDS

plasma; ultrafiltrate; protect MP from oxygen; comparison thin-layer Au/Hg electrode and HMDE

REFERENCE

Parsons,P.J.; LeRoy,A.F. Determination of cis-diamminedichloroplatinum(II) in human plasma using ion-pair chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *378*, 395–408.

SAMPLE

Matrix: blood
Sample preparation: Inject an aliquot of plasma ultrafiltrate.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil ODS coated with hexadecyltrimethylammonium bromide (Coat the column by passing 75 mL of a 27 mM aqueous solution of hexadecyltrimethylammonium bromide through the column at 30° at 1 mL/min (J.Chromatogr. 1981, 217, 405).)
Mobile phase: 10 mM pH 4.5 Citrate buffer containing 100 μM hexadecyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 290 following post-column reaction. The column effluent mixed with 26 μM potassium dichromate pumped at 0.1 mL/min and this mixture flowed through a 3.2 m × 0.3 mm i.d. knitted coil of PTFE tubing. The effluent from the coil mixed with 3.3 mM

sodium bisulfite solution pumped at 0.1 mL/min and this mixture flowed through a 44.6 m × 0.3 mm i.d. knitted coil of 0.3 mm i.d. PTFE tubing to the detector.

CHROMATOGRAM
Retention time: 9
Limit of detection: 40 ng/mL

KEY WORDS
plasma; ultrafiltrate; post-column reaction

REFERENCE
Marsh,K.C.; Sternson,L.A.; Repta,A.J. Post-column reaction detector for platinum(II) antineoplastic agents, *Anal.Chem.*, **1984**, *56*, 491–497.

SAMPLE
Matrix: blood
Sample preparation: Filter through a 10000 molecular mass cut-off filter (Filtron) by centrifuging at 4000 g at 4° for 30 min, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil SB strong anion exchanger
Mobile phase: MeOH:125 mM succinic acid adjusted to pH 5.2 with NaOH 60:40
Flow rate: 0.5
Detector: UV 344 following post-column derivatization with 20 mM sodium diethyldithiocarbamate in MeOH:water 60:40 pumped at 0.17 mL/min. The mixture flowed through a 500 × 3.5 mm packed-bed reactor packed with 75 μm silanized glass beads at 115° then to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: about 200 ng/mL

KEY WORDS
plasma; human; guinea pig; post-column reaction

REFERENCE
Andersson,A.; Ehrsson,H. Determination of cisplatin and cis-diammineaquachloroplatinum(II) ion by liquid chromatography using post-column derivatization with diethyldithiocarbamate, *J.Chromatogr.B*, **1994**, *652*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL MeCN, mix, centrifuge at 4° at 3500 g for 5 min. Remove a 200 μL aliquot of the supernatant and add it to 100 μL 10 mM citric acid containing 100 μM cetyltrimethylammonium bromide and 700 μL dichloromethane, rotate for 10 min, centrifuge at 4° at 3500 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Polymer RP (Brownlee)
Column: 100 × 4.6 BDS-Hypersil C18 (Before analyses pump 120 mL 50 mM cetyltrimethylammonium bromide in isopropanol:water 5:95 through the column at 30° to coat the column with cetyltrimethylammonium bromide.)
Mobile phase: 10 mM Citric acid containing 100 μM cetyltrimethylammonium bromide, adjusted to pH 5.0 with 5 M NaOH
Column temperature: 25
Flow rate: 0.7

Injection volume: 40
Detector: UV 290 following post-column reaction. The column effluent mixed with 117 μM potassium dichromate pumped at 0.2 mL/min and the mixture flowed through a 200 μL knitted coil of PTFE tubing at 30° and then mixed with 28.16 mM sodium bisulfite pumped at 0.2 mL/min. This mixture flowed through a 1 mL knitted coil of PTFE tubing at 30° to the detector. (The reaction coils were contained in a PCX 5000 Post Column Reaction Module (Pickering Laboratories, Mountain View CA).)

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 60 ng/mL

KEY WORDS
post-column reaction; plasma; dog; pharmacokinetics

REFERENCE
Farrish,H.H.; Hsyu,P.-H.; Pritchard,J.F.; Brouwer,K.R.; Jarrett,J. Validation of a liquid chromatography post-column derivatization assay for the determination of cisplatin in plasma, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 265–271.

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon 14 mm YMT-1 membrane) 1 mL plasma while centrifuging at 2000 g for 45 min. 500 μL Ultrafiltrate + 30 μL 100 μg/mL nickel chloride in 0.9% NaCl solution + 50 μL 10% sodium diethyldithiocarbamate in 100 mM NaOH (freshly prepared), heat at 37° for 1 h, cool, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil ODS C18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeOH:water 75:25
Column temperature: 30
Flow rate: 1.5
Injection volume: 100
Detector: UV 260 for 6.5 min then UV 250

CHROMATOGRAM
Retention time: 5.7
Internal standard: nickel chloride (7.4)
Limit of detection: 10 ng/mL
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Noninterfering: calcium, cobalt, copper, cyclophosphamide, etoposide, 5-fluorouracil, iron, lead, mitomycin, phosphorus, vinorelbine

KEY WORDS
plasma; ultrafiltrate; method does not distinguish between cisplatin and platinum-containing metabolites

REFERENCE
Augey,V.; Cociglio,M.; Galtier,M.; Yearoo,R.; Pinsani,V.; Bressolle,F. High-performance liquid chromatographic determination of cis-dichlorodiammineplatinum(II) in plasma ultrafiltrate, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1173–1178.

SAMPLE
Matrix: blood

Sample preparation: Filter (Amicon centriflow ultrafiltration membrane CF50, cut-off 50 000 Da) plasma while centrifuging at 4° at 1000 g for 10 min, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP18
Mobile phase: 15 mM pH 2.2 phosphoric acid
Flow rate: 1
Injection volume: 10-20
Detector: atomic absorption (Collect 15 s fractions of the HPLC effluent, analyze a 20 μL aliquot by atomic absorption with graphite furnace and deuterium background correction (Varian Model SpectrAA 300).)

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

rat; human; plasma; ultrafiltrate

REFERENCE

Bernareggi,A.; Torti,L.; Maffei Facino,R.; Carini,M.; Depta,G.; Casetta,B.; Farrell,N.; Spadacini,S.; Ceserani,R.; Tognella,S. Characterization of cisplatin-glutathione adducts by liquid chromatography-mass spectrometry. Evidence for their formation in vitro but not in vivo after concomitant administration of cisplatin and glutathione to rats and cancer patients, *J.Chromatogr.B*, **1995**, *669*, 247–263.

SAMPLE

Matrix: blood, formulations
Sample preparation: Whole blood. 5 mL Whole blood + 2 mL 10% trichloroacetic acid in water, mix thoroughly, let stand for 15 min, centrifuge for 20 min. Remove the supernatant and add it to 2 mL concentrated HCl, add 8 mL 10% trichloroacetic acid in water, mix, centrifuge for 15 min. Remove the supernatant and add it to 2 mL concentrated HCl, evaporate to near dryness with heat, dissolve the residue in 3 mL water, adjust the pH to 6, add 2 mL pH 8 sodium bicarbonate buffer, add 2 mL 1% bis(salicaldehyde)tetramethylethylenediamine in EtOH, warm for 15 min, cool, add 4 mL chloroform, mix well. Remove the chloroform layer and evaporate it to dryness, reconstitute with 100 μL MeOH, inject a 5 μL aliquot. Injections. Add 1 g cisplatin injection to 30 mL concentrated HCl, evaporate to dryness with heat, reconstitute with 10 mL water. Remove a 5 mL aliquot, add 2 mL pH 8 sodium bicarbonate buffer, add 2 mL 1% bis(salicaldehyde)tetramethylethylenediamine in EtOH, warm for 15 min, cool, add 4 mL chloroform, mix well. Remove a 2 mL aliquot of the chloroform layer and evaporate it to dryness, reconstitute with 1 mL EtOH, inject a 5 μL aliquot. (Preparation of bis(salicaldehyde)tetramethylethylenediamine is as follows. Stir 44.5 g 2-nitropropane and 84 mL 6 M NaOH with cooling, add 40 g bromine dropwise, add 165 mL EtOH, reflux gently for 3 h, pour into 500 mL ice-water, filter to obtain 2,3-dimethyl-2,3-dinitrobutane. Vigorously stir 17.6 2,3-dimethyl-2,3-dinitrobutane with 150 mL concentrated HCl at 50-60°, slowly add 75 g 20-mesh granular tin, reflux for 15 min, cool, make strongly alkaline with NaOH (Caution! Exothermic!), steam distil, collect 350 mL distillate. Add 100 g solid NaOH to the distillate to obtain 2,3-diamino-2,3-dimethylbutane as a separate layer (mp of oxalate 323-324°) (J. Am. Chem. Soc. 1955, 77, 6689). Stir 20 mmoles salicylaldehyde in 10 mL MeOH, add 10 mmoles 2,3-diamino-2,3-dimethylbutane in 6 mL MeOH dropwise, let stand for several h, collect the precipitate, recrystallize twice from MeOH to obtain bis(salicaldehyde)tetramethylethylenediamine as yellow needles (mp 117°) (Inorg. Chem. 1978, 7, 3389).)

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 30:50:20
Flow rate: 0.4
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: copper(II), iron(II), nickel(II), palladium(II), uranyl

KEY WORDS
derivatization; complexation; injections; whole blood

REFERENCE
Khuhawar,M.Y.; Lanjwani,S.N.; Memon,S.A. High-performance liquid chromatographic determination of cisplatin as platinum(II) in a pharmaceutical preparation and blood samples of cancer patients, *J.Chromatogr.B*, **1997**, *693*, 175–179.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter using a UFC 3GC membrane with a 10000 molecular weight cut-off (Japan Millipore) at 4000 g at 4° for 30 min, inject a 100 μL aliquot of the ultrafiltrate. Urine. Centrifuge at 1000 g for 1 min, dilute a 50 μL aliquot of the upper layer 1:10 with distilled water, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Cyano Guard-Pak (Waters)
Column: 150 × 4.6 5 μm anionic exchange resin (Hitachi No. 3013-N, Chromato Research)
Mobile phase: MeCN:10 mM NaCl 85:15
Column temperature: 40
Flow rate: 0.7
Injection volume: 100
Detector: UV 290 following post-column derivatization with 0.026 mM potassium dichromate at 0.14 mL/min and 6.6 mM sodium hydrogen sulfite at 0.07 mL/min using a 7000 × 0.5 mm or 30000 × 0.25 mm PTFE tube reactor.

CHROMATOGRAM
Retention time: 11
Limit of detection: 80 ng/mL

KEY WORDS
plasma; post-column reaction

REFERENCE
Kinoshita,M.; Yoshimura,N.; Ogata,H.; Tsujino,D.; Takahashi,T.; Takahashi,S.; Wada,Y.; Someya,K.; Ohno,T.; Masuhara,K.; Tanaka,Y. High-performance liquid chromatographic analysis of unchanged cis-diamminedichloroplatinum (cisplatin) in plasma and urine with post-column derivatization, *J.Chromatogr.*, **1990**, *529*, 462–467.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Direct injection. Plasma. Filter through a 10000 molecular mass cut-off membrane, inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS-2
Column: 150 × 4.6 5 μm OD5 C18 (Burdick & Jackson)
Mobile phase: 5 mM Sodium heptanesulfonate, 10% MeOH, 0.1% trifluoroacetic acid, pH 2.6
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: MS, Sciex Elan 250 ICP-MS coupled to the column with 960 × 0.1 mm PTFE capillary tubing, m/z 195 monitored, RF power 1.25 kW, nebulizer argon 0.9 L/min, auxiliary argon 1.2 L/min, coolant 12.5 L/min, spray chamber 5°.

CHROMATOGRAM

Retention time: 2.5
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Simultaneous: degradation products, hydrolysis products

KEY WORDS

plasma

REFERENCE

Zhao,Z.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of cisplatin and some possible metabolites by ion-pairing chromatography with inductively coupled plasma mass spectrometric detection, *J.Chromatogr.*, **1993**, *615*, 83–89.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon MPS-1 with a YMT membrane) while centrifuging at 3000 g at 4° for 15 min, inject an aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

HPLC VARIABLES

Column: 80 × 4.6 MCI gel CDR10
Mobile phase: MeCN:buffer 30:70 (Buffer was 100 mM sodium sulfate and 10 mM acetate, pH 5.5.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 290 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.3 mL/min, the mixture flowed through a 10 m × 0.5 mm i.d. coil of PTFE tubing held at 60° to the detector. The reagent was 40 mM sodium bisulfite and 10 mM acetate buffer, pH 5.5.

CHROMATOGRAM

Retention time: 10
Limit of detection: 20 nM

KEY WORDS

plasma; ultrafiltrate; rabbit; human; post-column reaction

REFERENCE

Kizu,R.; Yamamoto,T.; Yokoyama,T.; Tanaka,M.; Miyazaki,M. A sensitive postcolumn derivatization/UV detection system for HPLC determination of antitumor divalent and quadrivalent platinum complexes, *Chem.Pharm.Bull.*, **1995**, *43*, 108–114.

SAMPLE
Matrix: formulations
Sample preparation: Adjust pH to 7.0, dilute if necessary, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.2 5 μm Nucleosil C18
Mobile phase: 10 mM pH 7.0 phosphate buffer containing 0.55 mM hexadecyltrimethylammonium bromide (Condition column before use with 0.5% hexadecyltrimethylammonium bromide.)
Flow rate: 1
Detector: UV 216

CHROMATOGRAM
Retention time: 7
Limit of detection: 1000 ng/mL
Limit of quantitation: 5000 ng/mL

OTHER SUBSTANCES
Simultaneous: carboplatin

KEY WORDS
infusions; stability-indicating

REFERENCE
Rochard,E.; Boutelet,H.; Griesemann,E.; Barthes,D.; Courtois,P. Simultaneous high performance liquid chromatographic analysis of carboplatin and cisplatin in infusion fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 1505–1516.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 1.90

OTHER SUBSTANCES
Simultaneous: cimetidine (UV 228), dacarbazine (UV 300), granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

Sample preparation: Mix 2 mL of a 750 μg/mL solution of cisplatin in water with 1 mL 1.8 mg/mL o-phenylenediamine in water, heat at 100° for 20 min, filter (paper) the precipitate and wash it with water. Dissolve the precipitate in DMF and inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: Chloroform
Flow rate: 1
Injection volume: 100
Detector: UV 703

CHROMATOGRAM
Retention time: 3
Limit of detection: 400 ng/mL Pt

KEY WORDS
derivatization; complexation

REFERENCE
Hasson,H.; Warshawsky,A. High performance liquid chromatographic determination of *cis*-diammine-dichloroplatinum(II) (cisplatin) as the *o*-phenylenediamine complex, *J.Chromatogr.*, **1990**, *530*, 219–221.

SAMPLE
Matrix: solutions
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4 Silasorb SPH C18
Mobile phase: 4 mM sodium octanesulfonate + 6 mM tetrabutylammonium hydrogen sulfate + 20 mM KH_2PO_4, pH adjusted with concentrated NaOH to 5.9
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Macka,M.; Borák,J.; Semenková,L.; Kiss,F. Decomposition of cisplatin in aqueous solutions containing chlorides by ultrasonic energy and light, *J.Pharm.Sci.*, **1994**, *83*, 815–818.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb S porous graphitic carbon (Shandon)
Mobile phase: 1 mM NaOH
Column temperature: 0, 20
Flow rate: 0.5
Injection volume: 100

Detector: UV 283

CHROMATOGRAM
Retention time: 8.5 (0°), 6.5 (20°)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Ehrsson,H.C.; Wallin,I.B.; Andersson,A.S.; Edlund,P.O. Cisplatin, transplatin, and their hydrated complexes: Separation and identification using porous graphitic carbon and electrospray ionization mass spectrometry, *Anal.Chem.*, **1995**, *67*, 3608–3611.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize with four volumes saline at 4°, centrifuge at 100000 g at 4° for 1 h. Remove a 450 μL aliquot of the supernatant, filter (Millipore UFC 3GC 10000 molecular mass cut-off) with centrifuging at 4000 g at 4° for 30 min, inject a 20 μL aliquot of the supernatant

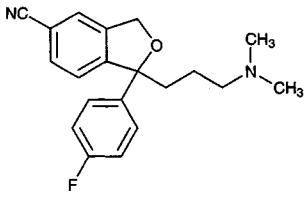
HPLC VARIABLES
Guard column: 50 × 4.6 5 μm Hitachi No. 3013-N
Column: 150 × 4.6 5 μm Hitachi No. 3013-N
Mobile phase: MeCN:10 mM NaCl 15:85
Column temperature: 40
Flow rate: 0.9
Injection volume: 100
Detector: UV 290 following post-column derivatization with 0.026 mM potassium dichromate at 0.6 mL/min and 6.6 mM sodium hydrogen sulfite at 0.3 mL/min using a 7000 × 0.5 mm or 30000 × 0.25 mm PTFE tube reactor.

CHROMATOGRAM
Retention time: 9
Limit of detection: 100 ng/g

KEY WORDS
rat; liver; kidney; ultrafiltrate; pharmacokinetics; post-column reaction; derivatization

REFERENCE
Hanada,K.; Nagai,N.; Ogata,H. Quantitative determination of unchanged cisplatin in rat kidney and liver by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 181–186.

Citalopram



Molecular formula: $C_{20}H_{21}FN_2O$
Molecular weight: 324.40
CAS Registry No.: 59729-33-8, 59729-32-7 (HBr)
Merck Index: 2379

SAMPLE

Matrix: blood
Sample preparation: Condition a 10 mL 100 mg Isolute C2 SPE cartridge (International Sorbent Technology) with 1 mL MeCN and 500 μL 25 mM pH 11.5 phosphate buffer. Add 1 mL MeCN:25 mM pH 11.5 phosphate buffer 5:95, 1 mL plasma, 100 μL 500 ng/mL IS, and 1 mL MeCN:25 mM pH 11.5 phosphate buffer 5:95 to the SPE cartridge reservoir. Suck the solution through the cartridge (15-35 kPa). Wash with 1 mL water, dry under vacuum for 5 min, wash with 1 mL MeCN, dry. Add 2 mL 1% acetic acid in MeOH, leave for 5 min, draw solvent through cartridge with vacuum (15-35 kPa). Evaporate eluate with nitrogen at 70°. Reconstitute with 100 μL mobile phase. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 μm Nova Pak phenyl
Column: 100 × 8 4 μm Nova Pak phenyl RCM radial compression
Mobile phase: MeCN:70 mmol/L phosphate buffer 40:60
Flow rate: 3
Injection volume: 20
Detector: F ex 235 em 290

CHROMATOGRAM

Retention time: 3.9
Internal standard: LU 10-202-O (5.8)
Limit of detection: 800 pM

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: alprazolam, amitriptyline, buspirone, clomipramine, clozapine, diazepam, desipramine, desmethylclomipramine, flunitrazepam, flupenthixol, haloperidol, hydroxyzine, imipramine, levopromazine, lofepramine, maprotiline, mianserin, nitrazepam, nortriptyline, oxazepam, paroxetine, perphenazine, propiomazin, thioridazine, zolpidem

KEY WORDS

plasma; SPE

REFERENCE

Carlsson,B.; Norlander,B. Solid-phase extraction with end-capped C2 columns for the routine measurement of racemic citalopram and metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 234–239.

SAMPLE

Matrix: blood
Sample preparation: Take 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:55:10
Flow rate: 0.6
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.8
Internal standard: citalopram

OTHER SUBSTANCES
Simultaneous: metapramine
Noninterfering: indalpine, diazepam, amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: carbamazepine

KEY WORDS
plasma; citalopram is IS

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: 1-2 mL Plasma + 50 μL 10 μg/mL desipramine in MeOH, make up to 3 mL with 100 mM NaOH, vortex for 1 min, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL diethyl ether. Add the eluate to 50 μL 5 mM phosphoric acid, evaporate under a stream of air at 40°. Add 1 mL diethyl ether to the residual solution, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak C18 guard-pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 41:50:9
Flow rate: 0.8
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 11.5
Internal standard: desipramine (14.5)
Limit of detection: 0.8 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: metoclopramide, oxazepam, dihydroergotamine, lorazepam, bromazepam, methotrimeprazine, cisapride, clobazam, diazepam, cyamemazine, alimemazine
Noninterfering: heptaminol, meprobamate, caffeine

KEY WORDS
plasma; SPE

REFERENCE

Rop,P.P.; Durand,A.; Viala,A.; Jorgensen,A. Simultaneous determination of citalopram, monodesmethylcitalopram and didesmethylcitalopram in plasma by high-performance liquid chromatography after column extraction, *J.Chromatogr.*, **1990**, *527*, 226–232.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 2 μL 10 μg/mL IS in water, inject on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A onto column B with mobile phase B, after 2 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES

Column: A 4 × 4 LiChrospher 100 RP-18; B 150 × 4.6 Ultron N-C18 (Shinwa Chemical Industries)

Mobile phase: A 1 mM pH 3.0 phosphate buffer; B MeCN:20 mM pH 4.6 phosphate buffer 30:70 containing 0.1% diethylamine

Column temperature: 40 (column B)

Flow rate: 1

Injection volume: 200

Detector: F ex 249 em 302

CHROMATOGRAM

Retention time: 20

Internal standard: 1-[3-(dimethylamino)propyl]-1-(4-chlorophenyl)-1,3-dihydro-5-isobenzofurancarbonitrile oxalate (H. Lundbeck Lu 10-202) (29)

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

column-switching; plasma; dog; rat; pharmacokinetics

REFERENCE

Matsui,E.; Hoshino,M.; Matsui,A.; Okahira,A. Simultaneous determination of citalopram and its metabolites by high-performance liquid chromatography with column switching and fluorescence detection by direct plasma injection, *J.Chromatogr.B*, **1995**, *668*, 299–307.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 1 μg/mL benzoctamine in 10 mM HCl + 100 μL 1 μg/mL desmethylbenzoctamine in 10 mM HCl + 50 μL 1 M NaOH + 6 mL heptane:isoamyl alcohol 98.5:1.5, shake for 15 min, centrifuge at 8° at 2100 g for 6 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 15 min, centrifuge at 8° at 2100 g for 15 min. Discard the organic phase and add 1 mL 1 M pH 9.4 sodium carbonate buffer to the aqueous phase, add 150 μL toluene:isoamyl alcohol 85:15, vortex for 15 min, centrifuge at 8° at 2100 g for 3 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 60 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm acetylated β-Cyclobond (Astec)

Mobile phase: MeCN:1% diethylamine 22:78 adjusted to pH 6.1 with acetic acid

Flow rate: 0.8

Injection volume: 50

Detector: F ex 240 em 296

CHROMATOGRAM
Retention time: 14.6 (S-(+)), 15.8 (R-(-))
Internal standard: benzoctamine (7.3), desmethylbenzoctamine (6.5)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral

REFERENCE
Rochat,B.; Amey,M.; Baumann,P. Analysis of enantiomers of citalopram and its demethylated metabolites in plasma of depressive patients using chiral reverse-phase liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 273–279.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 100 μL 1-2 μg/mL desipramine + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot. Tissue. Homogenize brain tissue gently (Ultra-Turrax) with two volumes 0.9% saline. 1 mL Homogenate + 100 μL 1-2 μg/mL desipramine + 1 mL 1 M NaOH + 250 mg NaCl + 6 mL dichloromethane, shake for 15 min, centrifuge at 1500 g for 15 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 100-150 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Spherisorb ODS C18
Mobile phase: MeCN:25 mM KH_2PO_4 50:50
Flow rate: 1.5
Injection volume: 100-150
Detector: UV 240

CHROMATOGRAM
Retention time: 9.3
Internal standard: desipramine (12.2)
Limit of detection: 25 ng/mL

KEY WORDS
plasma; rat; brain

REFERENCE
Wang,N.-S.; Lemmer,B. Determination of citalopram in plasma and brain tissue of the rat by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *488*, 492–497.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 Supelco C18
Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

CHROMATOGRAM

Retention time: 5.56
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline, nortriptyline
Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol
Noninterfering: benzodiazepines
Interfering: 8-hydroxyclomipramine

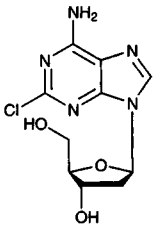
KEY WORDS

serum

REFERENCE

Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites, *J.Chromatogr.B*, **1996**, *675*, 83–88.

Cladribine



Molecular formula: $C_{10}H_{12}ClN_5O_3$
Molecular weight: 285.69
CAS Registry No.: 4291-63-8
Merck Index: 2397

SAMPLE

Matrix: blood
Sample preparation: Add 50 µL 1 µg/mL IS in MeOH:water 20:80 to 1 mL plasma, vortex for 2 s, add 500 µL 1 M sodium carbonate, add 7 mL ethyl acetate, vortex for 90 s, centrifuge at 2000 rpm for 5 min. Freeze in dry ice. Decant the organic layer and evaporate it to dryness under nitrogen, reconstitute with 100 µL MeOH:water 25:75, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: 20 mm long Supelcosil LC-8-DB
Column: 33 × 4.6 3 µm Supelcosil LC-8-DB
Mobile phase: MeOH:5 mM ammonium acetate 25:75
Flow rate: 1
Injection volume: 30
Detector: MS, PE-Sciex API IIIplus triple quadrupole, nebulizer probe 450°, nebulizing gas nitrogen at 80 psi, curtain gas UHP nitrogen at 1.5 L/min, corona discharge needle +3 µA, orifice potential +50 V, collision gas argon at 22 eV, m/z 286

CHROMATOGRAM

Retention time: 1.5
Internal standard: RWJ-29727 (6-amino-2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-9H-purine; R.W. Johnson, Raritan NJ) (m/z 304) (2.0)
Limit of quantitation: 0.1 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Moyer,M.D.; Johannsen,T.; Stubbs,R.J. Determination of 2-chlorodeoxyadenosine (cladribine, 2-CdA) in human plasma by liquid chromatography--atmospheric pressure chemical ionization mass spectrometry, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 45–51.

SAMPLE

Matrix: blood
Sample preparation: Add 200 µL ice-cold 400 mM perchloric acid containing 80 mM triethylammonium phosphate to the cell pellet (10^7-5 × 10^8 cells), vortex, add 100 µL ice-cold 1200 mM KOH containing 400 mM ammonium dihydrogen phosphate to reach pH 6.2. Vortex, centrifuge at 14500 g at 4° for 5 min, inject a 90 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: µBondapak C18 Guard-Pak
Column: 250 × 4.6 5 µm Ultrasphere ODS (Beckman Instruments)
Mobile phase: MeOH:80 mM pH 6.1 triethylammonium phosphate buffer 11:89
Flow rate: 1.5
Injection volume: 90
Detector: UV 265

CHROMATOGRAM
Retention time: 29.0

OTHER SUBSTANCES
Extracted: cladribine mono- di-, and triphosphates

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Reichelova,V.; Albertioni,F.; Liliemark,J. Determination of 2-chloro-2'-deoxyadenosine nucleotides in leukemic cells by ion-pair high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 115–123.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 125 pmole IS +5 mL ethyl acetate, vortex for 30 s, centrifuge at 700 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 40 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 mm long 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:MeOH:sodium phosphate buffer 5:10:85, pH 3.0
Flow rate: 1
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 5
Internal standard: 6-nitroimidazol-6-thioguanine (Guaneran) (5.5)
Limit of detection: 1 nM

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Liliemark,J.; Pettersson,B.; Juliusson,G. Determination of 2-chloro-2'-deoxyadenosine in human plasma, *Biomed.Chromatogr.*, **1991**, *5*, 262–264.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 5 mL acetone + 10 μL Brij 35 solution, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute with 1 mL mobile phase, add IS, inject a 50 μL aliquot.

HPLC VARIABLES
Column: LiChrosorb Si60
Mobile phase: MeOH:water:50 mM KH_2PO_4 5:45:50
Flow rate: 1
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Internal standard: 5,6-dimethylbenzo-1,2,4-triazole-1-β-D-ribofuranoside
Limit of detection: 1 μg/mL

KEY WORDS
serum

REFERENCE
Gajewska,M.; Pawinski,T. Determination of 2-chloro-2'-deoxyadenosine in human blood serum by high-performance liquid chromatography (Abstract 85), *Ther.Drug Monit.*, **1995**, *17*, 404–404.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in water at a concentration of 4 mg/mL, inject a 50-100 μL aliquot. Alternatively, dissolve in water at a concentration of 0.5 mg/mL, inject a 10 μL aliquot (for quantitative impurity profile).

HPLC VARIABLES
Guard column: 5 μm Rainin C18
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: Gradient. A was 100 mM ammonium acetate adjusted to pH 6.55 ± 0.1 with dilute acetic acid. B was MeCN. (a) A:B from 96:4 to 80:20 over 22 min, maintain at 80:20 for 12 min; or (b) (for late-eluting impurities), A:B from 89:11 to 50:50 over 21 min, maintain at 50:50 for 5 min; or (c) (for quantitative impurity profile) A:B from 96:4 to 80:20 over 22 min, maintain at 80:20 for 1 min, to 50:50 over 10 min, maintain at 50:50 for 20 min.
Flow rate: 1.2 ((a), (b)), 1 (c)
Injection volume: 10-100
Detector: UV 264 or MS, Finnigan MAT TSQ-70 triple quadrupole, thermospray, vaporizer 85°, ion source 250°, discharge 2000 V, scan 145-850, scan time 2 s

CHROMATOGRAM
Retention time: 21.5 (A), 7.5 (B)

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Weber,J.V.; Sampino,K.; Dunphy,R.; Burinsky,D.J.; Williams,T.; Motto,M.G. Characterization of cladribine and its related componds by high-performance liquid chromatography/mass spectrometry, *J.Pharm.Sci.*, **1994**, *83*, 525–531.

SAMPLE
Matrix: solutions

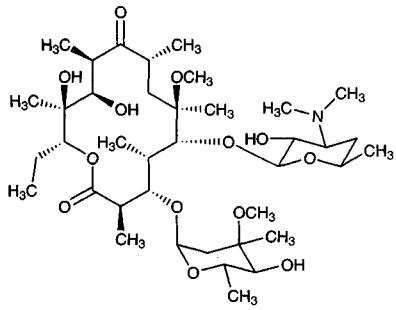
HPLC VARIABLES
Column: 80 × 4.6 3 μm (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 6.8 potassium phosphate buffer 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: fludarabine, analogs, degradation products

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Liquid chromatographic study of acid stability of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 711–714.

Clarithromycin



Molecular formula: $C_{38}H_{69}NO_{13}$
Molecular weight: 747.96
CAS Registry No.: 81103-11-9
Merck Index: 2400

SAMPLE

Matrix: blood
Sample preparation: Mix 200 µL plasma with 50 µL IS, add 200 µL 100 mM sodium carbonate, vortex for 30 s, add 3.5 mL MTBE, mix for 20 min, centrifuge at 2000 g for 10 min. Evaporate the MTBE layer to dryness at 37°, reconstitute the residue in 200 µL mobile phase, mix for 20 min, centrifuge, inject an 80 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 55:45
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem 5100 A, ESA 5010 dual electrode analytical cell at +680 mV and +780 mV, ESA 5020 guard cell +1.0 V

CHROMATOGRAM

Internal standard: azithromycin
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Patel,K.B.; Xuan,D.; Tessier,P.R.; Russomanno,J.H.; Quintiliani,R.; Nightingale,C.H. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2375–2379.

SAMPLE

Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 µL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng roxithromycin in MeOH:water 50:50 to 500 µL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor or 300 µL gastric mucosa homogenate, vortex, add 200 µL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 µL MeOH:water 50:50, inject a 20-50 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Zorbax SB CN

Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 37.5:6.3:56.2, pH 7.5 (The mobile phase was a mixture of 300 mL MeCN, 50 mL MeOH and 450 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 16
Internal standard: roxithromycin (18.5)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: azithromycin

KEY WORDS
pharmacokinetics; plasma; serum; leucocytes

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: gastric juice
Sample preparation: Dilute 500 μL gastric juice with 2.5 mL water, vortex for 1 min, filter (0.45 μm), inject a 50 μL aliquot.

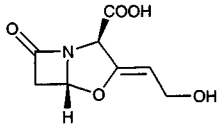
HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 50 mm pH 4.6 phosphate buffer containing 5 mM 1-octanesulfonic acid.)
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 5.8
Limit of detection: 400 ng/mL (water), 780 ng/mL (gastric juice)

OTHER SUBSTANCES
Extracted: degradation products

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Ion-pair high-performance liquid chromatographic assay method for the assessment of clarithromycin stability in aqueous solution and in gastric juice, *J.Chromatogr.B*, **1996**, *682*, 73–78.

Clavulanic acid



Molecular formula: $C_8H_9NO_5$
Molecular weight: 199.16
CAS Registry No.: 58001-44-8, 61177-45-5 (K salt)
Merck Index: 2402
Lednicer No.: 4 180

SAMPLE

Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 195

CHROMATOGRAM

Retention time: 2.71

OTHER SUBSTANCES

Simultaneous: granisetron (UV 300), ticarcillin

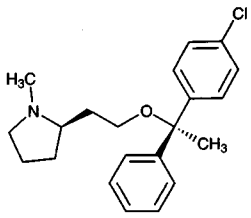
KEY WORDS

stability-indicating; injections; saline

REFERENCE

Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

Clemastine



Molecular formula: $C_{21}H_{26}ClNO$
Molecular weight: 343.90
CAS Registry No.: 15686-51-8, 14976-57-9 (fumarate)
Merck Index: 2405
Lednicer No.: 2 32

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 14.48

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, pen-

thienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 236

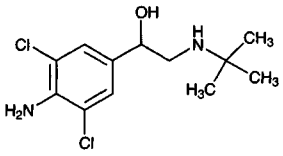
KEY WORDS

chiral; α = 3.04 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Clenbuterol



Molecular formula: $C_{12}H_{18}Cl2N_2O$
Molecular weight: 277.19
CAS Registry No.: 37148-27-9, 21898-19-1(monohydrochloride)
Merck Index: 2407

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 250 mM NaOH + 100 µL 50 ng/mL IS in water + 5 mL diethyl ether:2-butanol 90:10, shake for 10 min, centrifuge at 1500 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, vortex for 2 min, store at 4° overnight, warm to room temperature, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 7 µm LiChrosorb (select B) C18
Column: 125 × 4 7 µm LiChrosorb (select B) C18
Mobile phase: MeCN:buffer 23:77 containing 0.2 mM sodium 1-heptanesulfonate (Buffer was pH 4.0-4.1 phosphate buffer, ionic strength 0.1.)
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, Model 5011 detector, +0.75 V

CHROMATOGRAM

Retention time: 13.5
Internal standard: 4-amino-3,5-dichloro-α-[[(1,1-dimethylpropyl(amino]methyl]benzenemethanol (NAB 760) (10)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES

Extracted: mabuterol

KEY WORDS

horse; plasma

REFERENCE

Qureshi,G.A.; Eriksson,A. Determination of clenbuterol and mabuterol in equine plasma by ion-pair liquid chromatography with electrochemical detection. Chromatographic and electrochemical characteristics, *J.Chromatogr.*, **1988**, *441*, 197–205.

SAMPLE

Matrix: blood, urine
Sample preparation: Vortex 200 µL plasma or urine with 1 mL 1,2-dichloroethane for 10 min and centrifuge for 10 min. Transfer the organic layer to a clean glass tube, evaporate to dryness under nitrogen, redissolve the residue in 200 µL mobile phase, filter, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 Chirex 3022 (chiral stationary phase made of (S)-indoline-2-carboxylic acid and (R)-1-(α-naphthyl)ethylamine) (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:ethanol/trifluoroacetic acid 80:10:10 (Ethanol and trifluoroacetic acid were pre-mixed at a ratio of 20:1.)
Column temperature: 23
Flow rate: 0.8
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 9.88 (+), 11.77 (-)
Limit of quantitation: 100 .pico.M

KEY WORDS
plasma; chiral

REFERENCE
Abou-Basha,L.I.; Aboul-Enein,H.Y. Direct enantioselective separation of clenbuterol by chiral HPLC in biological fluids, *Biomed.Chromatogr.*, **1996**, *10*, 69–72.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 10.802

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 13 mM Empore C18 SPE disk (Baker) with 2.5 mL MeOH and 2.5 mL water at 1.5 mL/min. Dissolve tablet in dissolution medium (?). Pass 50 mL through the SPE disk, wash with 2.5 mL water, dry, add 1 mL MeOH and let it soak in for 3 min, elute at 0.5 mL/min, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher cyano

Mobile phase: MeOH:10 mM pH 6.6 phosphate buffer 75:25
Column temperature: 25
Flow rate: 1.3
Injection volume: 50
Detector: UV 214

KEY WORDS
tablets; SPE; comparison with capillary electrophoresis

REFERENCE
Carducci,C.N.; Lucangioli,S.E.; Rodríguez,V.G.; Fernández Otero,G.C. Application of extraction disks in dissolution tests of clenbuterol and levothyroxine tablets by capillary electrophoresis, *J.Chromatogr.A*, **1996**, *730*, 313–319.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 11.8

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: pyrithyldione

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)

Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 248

KEY WORDS
chiral; α = 1.27 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)
Mobile phase: Isopropanol:15 mM pH 5.0 acetate buffer 1:99
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 3.5, 5 (enantiomers)

KEY WORDS
chiral

REFERENCE
Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE
Matrix: tissue
Sample preparation: Add 5 mL 1 M HCl to the tissue, vortex for 1 min, sonicate for 10 min, and centrifuge at 2100 g for 20 min. Remove the supernatant and add 5 mL 1 M EDTA in 4 M NaOH. Adjust the pH to 12.2 ± 0.1 with 10 M NaOH, keep at room temperature for 2 h. Add 5 mL diethyl ether to the sample, vortex for 1 min, shake for 4 min, and centrifuge at 2100 rpm for 2 min. Remove the diethyl ether layer and dry it under nitrogen at 80°. Add another 5 mL of diethyl ether to the aqueous phase, vortex, centrifuge, and add the ether layer to the same vial that contains the residue from the previous extraction. Dry under nitrogen at 80°, dissolve the residue in 200 μL of 1% formic acid, vortex for 5 min, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18e
Column: 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: MeOH:water:formic acid 34:66:1
Column temperature: 40
Flow rate: 1.0
Injection volume: 50
Detector: E, Hewlett Packard Model 1049A, glassy carbon electrode 1.3V for 999 ms pulsed to -2.0V for 200 ms (to prevent fouling electrode), solid state Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8

Limit of detection: 5 ng/mL

KEY WORDS
retina; cow

REFERENCE
Lin,L.A.; Tomlinson,J.A.; Satzger,R.D. Detection of clenbuterol in bovine retinal tissue by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *762*, 275–280.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg WCX-SPE weak cation-exchange SPE cartridge (Baker) with 10 mL EtOH, 3 mL water, 3 mL 100 mM pH 6 sodium dihydrogen phosphate, and 3 mL water. Wash a RidaScreen immunoaffinity (IA) cartridge (Bioman Products, Canada) with 2 mL pH 7.4 phosphate buffered saline (PBS). Add 30 mL 10 mM HCl to 6 g tissue, shake for 20 s, sonicate for 15 min. Shake and heat at 80° for 30 min. Cool at -15°, centrifuge at 9200 g at 5° for 20 min. Adjust the pH of the supernatant to 6 with 1 M NaOH. Add to the SPE cartridge, wash with 4 mL water and 4 mL EtOH. Elute with 5 mL 2% ammonium hydroxide in EtOH. Evaporate eluate to 100 μL under a stream of nitrogen at 30°. Dilute to 1 mL with PBS. Add 1 mL to the IA cartridge, wash with 500 μL PBS, wash with 1 mL EtOH:water 20:80. Elute with 2 mL EtOH:water 80:20. Evaporate the eluate to 100 μL under a stream of nitrogen at 30°, dilute to 400 μL with mobile phase B, mix. Inject a 150 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeOH. B was water containing 10 mM acetic-acid ammonium acetate buffer adjusted to pH 4.6 with acetic acid. A:B from 30:70 to 70:30 over 5 min
Flow rate: 0.8
Injection volume: 150
Detector: UV 245

CHROMATOGRAM
Retention time: 4.6
Limit of detection: 300 pg/g

KEY WORDS
cow; liver; muscle; SPE; immunoaffinity

REFERENCE
Lawrence,J.F.; Ménard,C. Determination of clenbuterol in beef liver and muscle tissue using immunoaffinity chromatographic cleanup and liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *696*, 291–297.

SAMPLE
Matrix: urine
Sample preparation: Add ammonia to 20 mL urine to adjust the pH to ca. 11. Add the sample to a Chem Elut SPE cartridge until completely absorbed. After 10 min elute with three 20 mL portions of n-hexane, evaporate the eluate to dryness. Redissolve in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Nova-Pak C18
Column: 150 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeOH:3.5mM pH 3.0 $(NH_4)_2HPO_4$ 90:10
Flow rate: 1.5

Detector: UV 246

CHROMATOGRAM

Retention time: 3.9
Limit of detection: 1 ng/mL

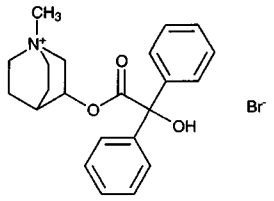
KEY WORDS

comparison with GC-MS/EI/SIM and ELISA; cow; SPE

REFERENCE

Ramos,F.; Castilho,M.C.; Noronha Da Silveira,M.I. Occurrence of β2-adrenergic agonist residues in urine of animal meat producers in Portugal, *J.AOAC Int.*, **1998**, *81*, 544–548.

Clidinium bromide



Molecular formula: $C_{22}H_{26}BrNO_3$
Molecular weight: 432.36
CAS Registry No.: 3485-62-9
Merck Index: 2412

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.27

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Open capsules, weigh out amount equivalent to 5 mg clidinium bromide, add 15 mL water, sonicate for 10 min with gentle swirling. Make up to 25 mL with water, centrifuge, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 µm Partisil 10 ODS-3
Mobile phase: MeCN:300 mM $(NH_4)H_2PO_4$ 32:68, adjust pH to 4.3 ± 0.1 with 10% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 235

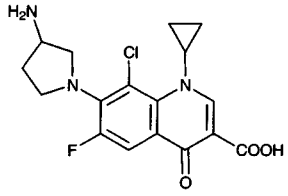
CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, impurities

KEY WORDS
capsules

REFERENCE
Yuen,S.M.; Lehr,G. Liquid chromatographic determination of clidinium bromide and clidinium bromide-chlordiazepoxide hydrochloride combinations in capsules, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 461–464.

Clinafloxacin



Molecular formula: $C_{17}H_{17}ClFN_3O_3$
Molecular weight: 365.79
CAS Registry No.: 105956-97-6, 105956-99-8 (HCl)
Merck Index: 2413

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 25 μL water + 25 μL 15 μg/mL IS + 50 μL MeCN: perchloric acid 80:20, centrifuge at 10687 g for 10 min, inject a 150 μL supernatant aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm BDS-Hypersil C18
Mobile phase: MeCN:buffer 20:80, (Buffer (ion pairing solution) was 50 mM citric acid containing 1.15 mM tetrabutylammonium hydroxide and 0.1% ammonium perchlorate, adjusted to pH 4.0.)
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 6
Internal standard: 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-[3-[(methylamino)methyl]-1-pyrrolidinyl]-4-oxo-3-quinolinecarboxylic acid (PD 118012) (7.9)
Limit of quantitation: 25 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Brodfuehrer,J.I.; Priebe,S.; Guttendorf,R. Achiral and chiral high-performance liquid chromatographic methods for clinafloxacin, a fluoroquinolone antibacterial, in human plasma, *J.Chromatogr.B*, **1998**, *709*, 265–272.

SAMPLE

Matrix: blood
Sample preparation: Condition a C18 SPE cartridge with two 1 mL portions of MeCN, four 1 mL portions of MeCN:water 50:50, and two 1 mL portions of water. 1 mL Plasma + 100 μL water + 50 μL 20 μg/mL IS + 1 mL 0.25 mM decylamine in water, mix, add to the SPE cartridge, wash with 1 mL MeCN:water 5:95, elute with 1 mL MeOH:water 40:60, evaporate under nitrogen at 45°, reconstitute the residue in 200 μL MeOH:water 15:85, inject a 125 μL aliquot. (Protect from light!)

HPLC VARIABLES

Column: 150 × 4 5 μm Daicel Crownpak CR(+) (Chiral Technologies, Exton, PA)
Mobile phase: MeOH:water 12:88 containing 0.1 mM decylamine, adjusted to pH 2.0 with perchloric acid
Column temperature: 35
Flow rate: 1
Injection volume: 125
Detector: UV 340

CHROMATOGRAM

Retention time: 31.3 ± 1.0 (R), 36.9 ± 1.0 (S)

Internal standard: R-7-[3-(1-amino-1-methyl-ethyl)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid, monohydrochloride (PD 138312) (54.7 ± 1.0)

Limit of quantitation: 40 ng/mL

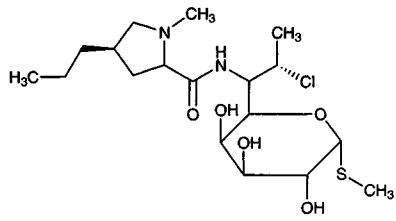
KEY WORDS

plasma; chiral; SPE; pharmacokinetics; protect from light

REFERENCE

Brodfuehrer,J.I.; Priebe,S.; Guttendorf,R. Achiral and chiral high-performance liquid chromatographic methods for clinafloxacin, a fluoroquinolone antibacterial, in human plasma, *J.Chromatogr.B*, **1998**, *709*, 265–272.

Clindamycin



Molecular formula: $C_{18}H_{33}ClN_2O_5S$

Molecular weight: 424.99

CAS Registry No.: 18323-44-9, 58207-19-5 (HCl monohydrate), 36688-78-5 (palmitate), 25507-04-4 (palmitate HCl), 24729-96-2 (phosphate)

Merck Index: 2414

SAMPLE

Matrix: blood

Sample preparation: Prepare a silica SPE cartridge. Fill a 3 mL cartridge with 500 mg Silica gel 60 (Merck). Condition it with 2.5 mL MeOH and with 2.5 mL water. Spike 500 μL plasma or serum with 30 μL 6 ng/μL propranolol, add to the SPE cartridge. Wash with 1 mL water, elute with 3 mL MeOH (added dropwise). Evaporate eluates to dryness under a gentle stream of nitrogen. Reconstitute the residue in 200 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Keystone ODS2

Mobile phase: MeCN:THF:50 mM pH 5.00 phosphate buffer 24:1:75

Flow rate: 1

Injection volume: 30

Detector: UV 204

CHROMATOGRAM

Retention time: 12

Internal standard: propranolol (21)

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; serum; SPE; pharmacokinetics

REFERENCE

Liu,C.-M.; Chen,Y.-K.; Yang,T.-H.; Hsieh,S.-Y.; Hung,M.-H.; Lin,E.T. High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections, *J.Chromatogr.B*, **1997**, *696*, 298–302.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.962

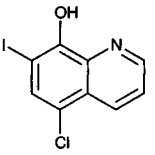
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Clioquinol



Molecular formula: C_9H_5ClINO
Molecular weight: 305.50
CAS Registry No.: 130-26-7
Merck Index: 5052

SAMPLE

Matrix: formulations
Sample preparation: 1 g Ointment + 30 mL trimethylpentane, warm on a water bath until ointment melts, add 10 mL 4% bromobenzene in MeOH:water 80:20, extract with 30 mL methanol:50 mM phosphoric acid 80:20 then twice with 20 mL methanol:50 mM phosphoric acid 80:20, combine extracts, cool, make up to 100 mL with methanol:50 mM phosphoric acid 80:20, inject 20 μL aliquot.

HPLC VARIABLES

Column: 225 × 4 Hypersil-ODS
Mobile phase: MeOH:50 mM phosphoric acid 80:20
Flow rate: 2
Injection volume: 20
Detector: UV 256

CHROMATOGRAM

Retention time: 5
Internal standard: bromobenzene (3.5)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: hydrocortisone

KEY WORDS

ointment

REFERENCE

Phoon,K.W.; Stubley,C. Rapid method for the simultaneous analysis of hydrocortisone and clioquinol in topical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *246*, 297–303.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out ointment or cream containing 30 mg clioquinol, add 50 mL THF, warm on steam bath to dissolve, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 100 mg/mL nickel(II) chloride hexahydrate in MeOH, add 5 mL 400 μg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm phenyl (Waters)
Mobile phase: MeCN:MeOH:water 27:18:55 containing 1 mM nickel chloride and 10 mM ammonium acetate
Flow rate: 1.2-1.5
Injection volume: 10-20
Detector: UV 273

CHROMATOGRAM

Retention time: 7.5

Internal standard: diphenylamine (11.5)

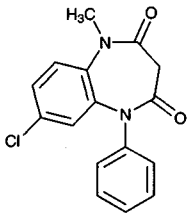
KEY WORDS

ointment; cream

REFERENCE

Wojtowicz,E.J. Reverse-phase liquid chromatographic determination of clioquinol in cream and ointment preparations: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 562–563.

Clobazam



Molecular formula: $C_{16}H_{13}ClN_2O_2$
Molecular weight: 300.74
CAS Registry No.: 22316-47-8
Merck Index: 2417
Lednicer No.: 2 406

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 4.00

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 229.9

CHROMATOGRAM

Retention time: 19.19

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4.6 10 μm LiChrosorb RP8

Mobile phase: MeOH:100 mM pH 7.0 tetraethylammonium phosphate buffer 39:48
Flow rate: 2
Injection volume: 75
Detector: UV 230 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector., F ex 364 em 400 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector.

CHROMATOGRAM

Retention time: 5
Limit of detection: 1 ng

OTHER SUBSTANCES

Extracted: metabolites

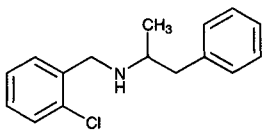
KEY WORDS

post-column reaction; post-column photochemical derivatization

REFERENCE

Uihlein,M.; Schwab,E. A novel reactor for photochemical post-column derivatization in HPLC, *Chromatographia*, **1982**, *15*, 140–146.

Clobenzorex



Molecular formula: $C_{16}H_{18}ClN$
Molecular weight: 259.78
CAS Registry No.: 13364-32-4, 5843-53-8 (HCl)
Merck Index: 2421

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.912

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 2 mg of the hydrochloride in 30 mL 450 mM NaOH, extract with 30 mL chloroform. Add 10 µL phenylisothiocyanate to the chloroform solution, evaporate to dryness under a stream of air, reconstitute with 1 mL MeOH, inject a 5 µL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 µBondapak C18
Mobile phase: MeOH:water:acetic acid 65:34:1
Flow rate: 1.5
Injection volume: 5

Next Page

Detector: UV 254

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Simultaneous: 3- and 4-chloro analogs

KEY WORDS

derivatization

REFERENCE

Noggle,F.T.,Jr.; Clark,C.R.; Andurkar,S.V.; DeRuiter,J. Liquid chromatographic analysis of regioisomers and enantiomers of N-(chlorobenzyl)-α-methylphenethylamines: Analogues of clobenzorex, *J.Liq.Chromatogr.*, **1990**, *13*, 763–777.